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Altered Tropism of an Ovine Adenovirus Carrying the Fiber Protein Cell Binding Domain of Human Adenovirus Type 5

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Ovine adenovirus OAV287 (OAV) is phylogenetically and serotypically distinct from human Ad5. OAV grows productively in CSL503 foetal ovine lung cells and abortively infects several human cell lines. OAV has a unique fiber and a penton protein that lacks a recognisable integrin-binding motif. It is not known whether a secondary receptor is required for infection. A hybrid virus was constructed in which the cell binding domain on the OAV fiber protein was exchanged for the equivalent region from human adenovirus type 5. The hybrid OAV grew to titres that were 1 to 2 $\log_{10}$ lower than wild-type OAV in permissive ovine cells. Human Ad5 also infected CSL503 cells but failed to compete with OAV for receptor binding sites on those cells. However, the hybrid virus did compete with Ad5, consistent with its use of the Ad primary receptor. The hybrid virus was also neutralised by Ad5 antiserum whereas OAV was not. Human 293 kidney and LNCaP prostate cell lines that were not detectably infected by OAV were infected by the hybrid virus and other human prostate and breast cancer cell lines showed greatly enhanced infectivity. Thus, modification of the fiber cell binding domain was sufficient to profoundly alter the tropism of OAV, suggesting that the interaction between the primary receptor and the virus particle is the major factor controlling virus entry during infection.

INTRODUCTION

Human adenoviruses infect a wide variety of cell types by what is generally thought to be a two-step process. Binding of virus particles to the cell surface usually occurs by an interaction between the cell binding domain (cbd) located at the tip of the trimeric fiber protein (Philipson *et al.* 1968; Henry *et al.,* 1994; Xia *et al.,* 1994) and a primary cellular receptor. After binding, secondary interactions between an Arg/Gly/Asp (RGD) motif in the penton base protein and cellular integrins are thought to enhance internalisation of particles via endocytosis (Bai *et al.,* 1993; Wickham *et al.,* 1993). However, there are now numerous variations to this theme. In the context of hematopoietic cells, interactions between the penton base protein and distinct integrins alone can mediate Ad2 binding and entry, β2 integrin acting as the primary receptor with α_v integrin mediating uptake (Huang *et al.,* 1996). Similarly, direct interaction of Ad9 with α_v integrin facilitates virus uptake (Roelvink *et al.,* 1996). In other circumstances where the RGD motif is absent, an integrin-independent pathway that is receptor concentration-dependent appears to operate. Multiple attachment points between fiber and a cellular receptor may be utilised to promote virus uptake (Freimuth, 1996). Thus, in particular cell types, different proteins can serve as attachment points for adenoviruses that bind *via* the fiber or penton protein. The combination of interactions between viral capsid proteins and the cellular receptors determines the tropism of the virus. Understanding these interactions and how they operate for particular viruses will be important for the design of viral vectors with the ability to target specific cell types.

In contrast to human Ads, cell binding and uptake of non-human Ads has not been well studied. No primary receptor has been identified and because RGD motifs are altered or missing (Bai *et al.,* 1993; Vrati *et al.,* 1996a), the involvement of secondary receptors in uptake remains to be determined. Conceivably, other capsid sequences could be utilised for secondary interactions or viruses may enter cells *via* the primary receptor, as suggested for RGD-negative human Ads (Freimuth, 1996). We are investigating an ovine adenovirus OAV287 (OAV), which is the best studied member of a new group of adenoviruses that are phylogenetically distinct from the *Mastadenoviruses* and *Aviadenoviruses* (Harrach *et al.,* 1997). OAV grows well in a foetal ovine lung cell line and, to a limited extent, in bovine nasal turbinate cells (Boyle *et al.,* 1994; Khatri *et al.,* 1997). The OAV fiber is predicted to contain 25 repeating units and has a unique C-terminal cbd of 121 residues (Vrati *et al.,* 1995) that is smaller than the cbd of Ad5 (185 residues), suggesting that OAV recognises a different receptor. The OAV penton shows 55% identity with Ad2 penton, but the sequences corresponding to the RGD domain are missing (Vrati *et al.,* 1996a). Despite its distinctive cell attachment proteins, OAV infected several human cell lines, albeit with differ-

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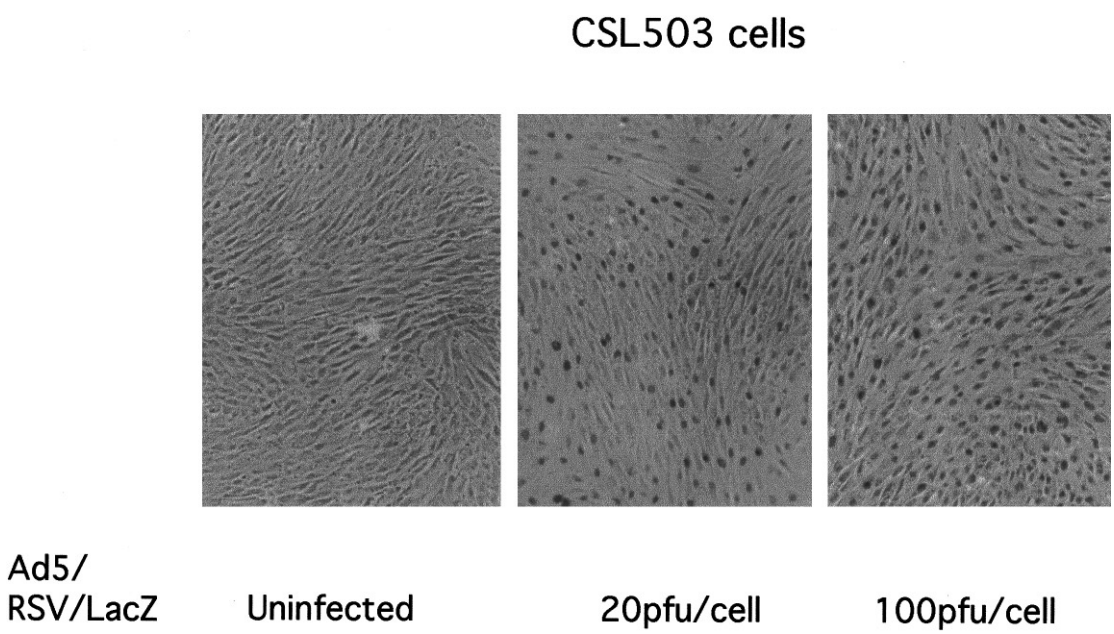

FIG. 1 Ovine foetal lung fibroblasts are infected by a human Ad5 recombinant. CSL503 cells were infected with 0, 20, or 100 pfu/cell of Ad5/RSV/lacZ and stained for β-galactosidase expression at 24 h p.i.

ing efficiencies. LNCaP prostate cancer and 293 human embryonic kidney cells were not detectably infected, as assayed by transgene expression and reverse transcription-polymerase chain reaction (RT-PCR) analysis (Khatri *et al.,* 1997). In an effort to modify the cell tropism of OAV and to begin to investigate its process of cell entry, we constructed a hybrid virus in which the OAV cbd on fiber was replaced by the larger cbd from Ad5 (Henry *et al.,* 1994). The effect of this change on the growth properties and host range of OAV were investigated.

RESULTS

CSL503 cells are infected by Ad5

As a prerequisite for rescuing a hybrid OAV carrying the Ad5 cbd, it was necessary to identify a cell line that was both permissive for OAV and carried an appropriate receptor(s) for Ad5. Because CSL503 cells are one of the few lines that are permissive for OAV (Boyle *et al.,* 1994; Khatri *et al.,* 1997), the ability of Ad5 to infect those cells was investigated using an Ad5/RSV/lacZ recombinant. Cells were infected at an m.o.i. of 20 to 100 plaque forming units (pfu)/cell and stained for β-galactosidase expression after 24 h p.i. Over this range of multiplicities 50 to 90% of the cells were infected (Fig. 1).

Construction and characterisation of hybrid virus OAV206f

Plasmid pOAV206f was constructed and used to rescue the corresponding virus (Fig. 2). Transfection of the plasmid into CSL503 cells produced a cytopathic effect after 20 days. This was slower than generally observed for other rescued viruses (Vrati *et al.,* 1996b; Khatri *et al.,* 1997; Xu *et al.,* 1997). Digestion of the viral genome with *Cel*II produced the expected fragments of 26 and 5.6 kb while digestion with *Nco*I produced fragments of 9.1, 6.3, 5.5, 3.3, 2.3, 2.0, 1.4, and 1.3 kb (fragments of 0.34 and 0.06 kb were not visible), as predicted from the sequence (data not shown).

Infectious stocks of OAV206f were grown by infecting CSL503 cells and allowing cpe to progress to completion. When CSL503 cells were infected at 1 pfu/cell with OAV, OAV206, or OAV206f, complete cpe developed in about 4 days for the first two viruses but took about 6 days for the hybrid virus. Under these conditions the hybrid virus grew to a titer which was one to two $\log_{10}$

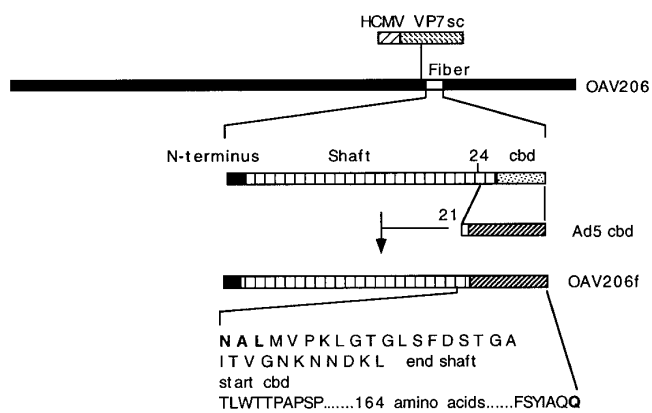

FIG. 2. Construction of an OAV/Ad5 hybrid virus by exchange of cell binding domains. The hybrid fiber protein contains repeats 21 and 22 plus the cbd of Ad5 fused to repeat 24 of OAV fiber. OAV sequences are shown in bold.

TABLE 1

Neutralisation of Ad5, OAV and OAV206f with Polyclonal Antisera

	Virus		
	OAV206	Ad5	OAV206f
Antiserum			
Ad5	<1:40	1:2560	1:1280
OAV	1:2560	<1:40	1:2560
Normal ovine	<1:20	<1:20	<1:20

units lower than obtained for other recombinant viruses. Titres in the range of 5×10^7–1×10^8 pfu/ml were obtained.

Neutralisation of OAV, Ad5, and OAV206f

A comparison of the capsid protein sequences of Ad5 and OAV predicted that the two viruses should be serotypically distinct (Vrati *et al.,* 1996a) but Ad5 antiserum was expected to neutralise OAV206f because it carries the Ad5 cbd. To confirm this, duplicate aliquots containing 50 pfu of OAV, Ad5, or OAV206f were incubated at room temperature for 1.5 h with serial twofold dilutions of polyclonal OAV or Ad5 antisera. CSL503 cells were then infected with pre-incubated OAV or hybrid virus while 293 cells were infected with pre-incubated Ad5. Cells were incubated for 7 to 14 days to allow a complete cpe to develop. The data (Table 1) show that none of the viruses was neutralised by serum from uninfected sheep. OAV and Ad5 were each neutralised by the homologous serum to a dilution of 2560, but neither virus was neutralised by the reciprocal antiserum even at a dilution of 1:40. This confirmed that OAV is serotypically distinct from at least one common human adenovirus. OAV206f and wild-type OAV were neutralised by the OAV antiserum at the same dilution because the two viruses had common hexon and penton proteins that are major viral antigens. However, OAV206f was also neutralised by the Ad5 antiserum, albeit at a slightly lower dilution than Ad5 (Table 1), confirming that it carried the Ad5 cbd.

OAV206f infects CSL503 cells via an Ad5 receptor

Competitive binding experiments were carried out to investigate whether OAV and Ad5 bind to cells via distinct receptors. To allow binding but to prevent uptake CSL503 cells were incubated at 4°C. Radiolabelled Ad5 was incubated with cells in the presence of increasing amounts of unlabelled Ad5, or OAV216, a recombinant virus that carried an HCMV/alkaline phosphatase reporter cassette (Khatri *et al.,* 1997) but that had an unmodified fiber protein. Binding of radiolabelled Ad5 was reduced by competition with unlabelled Ad5 but no significant competition was observed with OAV216 (Fig. 3A), showing that these viruses recognised distinct primary receptors.

Similarly, binding of radiolabelled OAV206f to CSL503 cells was monitored in the presence of increasing amounts of OAV216, Ad5, or homologous virus. Competition with OAV206f was observed, as expected, with a ~50% reduction in binding observed at an input of 100 pfu/cell. However, at this input there was no reduction with OAV216 ($P < 0.05$) (Fig. 3B). At an input of 1000 pfu/cell OAV206f homologous competition reduced binding by ~70%, however, OAV216 produced a reduction of only ~25% ($P < 0.05$) (Fig. 3B). At both viral inputs, Ad5 competed equally well with OAV206f. This confirmed that Ad5 and OAV206f shared a receptor that was distinct from the receptor for wild-type OAV.

Altered cell tropism of OAV206f

The ability of OAV206f to infect various cell types was compared with that of the parental OAV206 (which carried the identical gene cassette) using equal inputs of virus for infection. Virus preparations were titred by

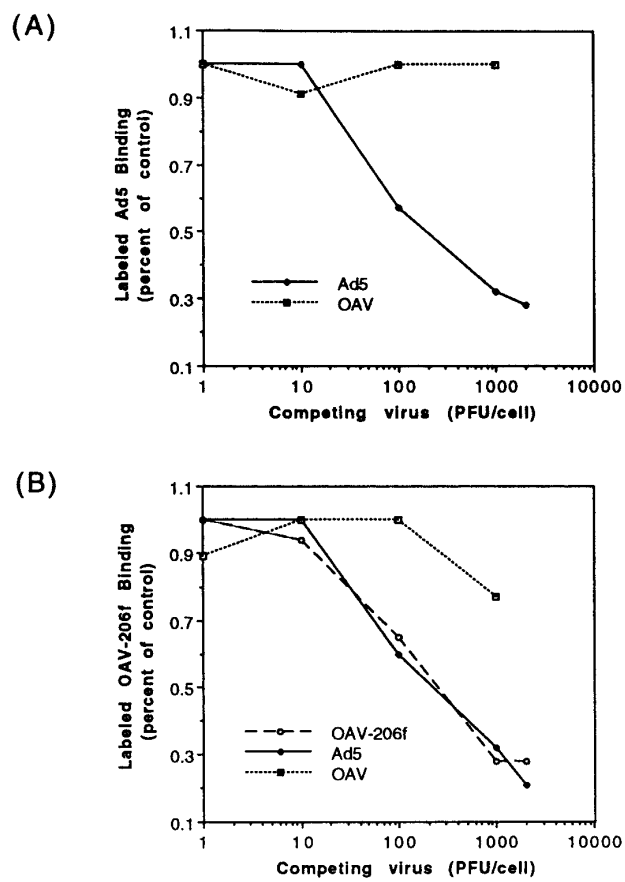

FIG. 3. Competitive binding of viruses to CSL503 cells. Radiolabelled Ad5 (A) or OAV206f (B) was bound to CSL503 cells at 4°C in the presence of increasing amounts of unlabelled Ad5, OAV206f, or OAV216, as indicated. OAV216 carries a wild-type fiber cbd. Data points represent the means of duplicate samples. Standard *t* tests were used to analyse the statistical significance of the data (see text).

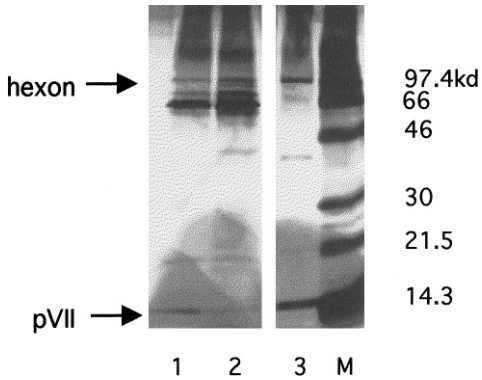

FIG. 4. OAV206 and OAV206f preparations contain similar particle numbers. An equal number of pfu of OAV206f (lane 1) and OAV206 (lane 2) were analysed by SDS-PAGE together with a purified, unrelated OAV recombinant (lane 3). The positions of previously described hexon and VII proteins (Vrati *et al.,* 1996a) on the silver stained gel are indicated.

plaque assay, and an equal number of pfus were analysed by SDS-PAGE to determine viral particle levels. Although not pure, it was apparent that each virus preparation contained a similar number of particles based on the relative amounts of structural proteins, e.g., hexon and pVII, that were resolved (Fig. 4, lanes 1 and 2). Permissive CSL503 cells were then infected with OAV206 or OAV206f at an m.o.i. of 10 pfu/cell. Expression of VP7sc was monitored by radioimmuno-precipitation at 12, 24, and 36 h p.i. The kinetics and level of expression of the reporter by the two viruses was essentially identical over this time frame (Fig. 5A, lanes 3, 4, 6, and 7).

Approximately equal numbers of nonpermissive cells were then infected with the two viruses at an m.o.i. of 10 pfu/cell. Although OAV206 is known to infect and express VP7sc in several of the cell types used below (Khatri *et al.,* 1997), under the conditions of analysis used in these experiments VP7sc expression was not detectable at 12 to 36 h p.i. in OAV206-infected MCF-7 cells (Fig. 5A, lanes 9 to 11) or at 12 to 48 h p.i. in LNCaP (Fig. 5B, lanes 2 to 4), PC-3 (Fig. 5B, lanes 9 to 11), or 293 cells (Fig. 5C, lanes 2 to 4). However, after infection with OAV206f, VP7sc was prominently expressed in MCF-7, LNCaP, and

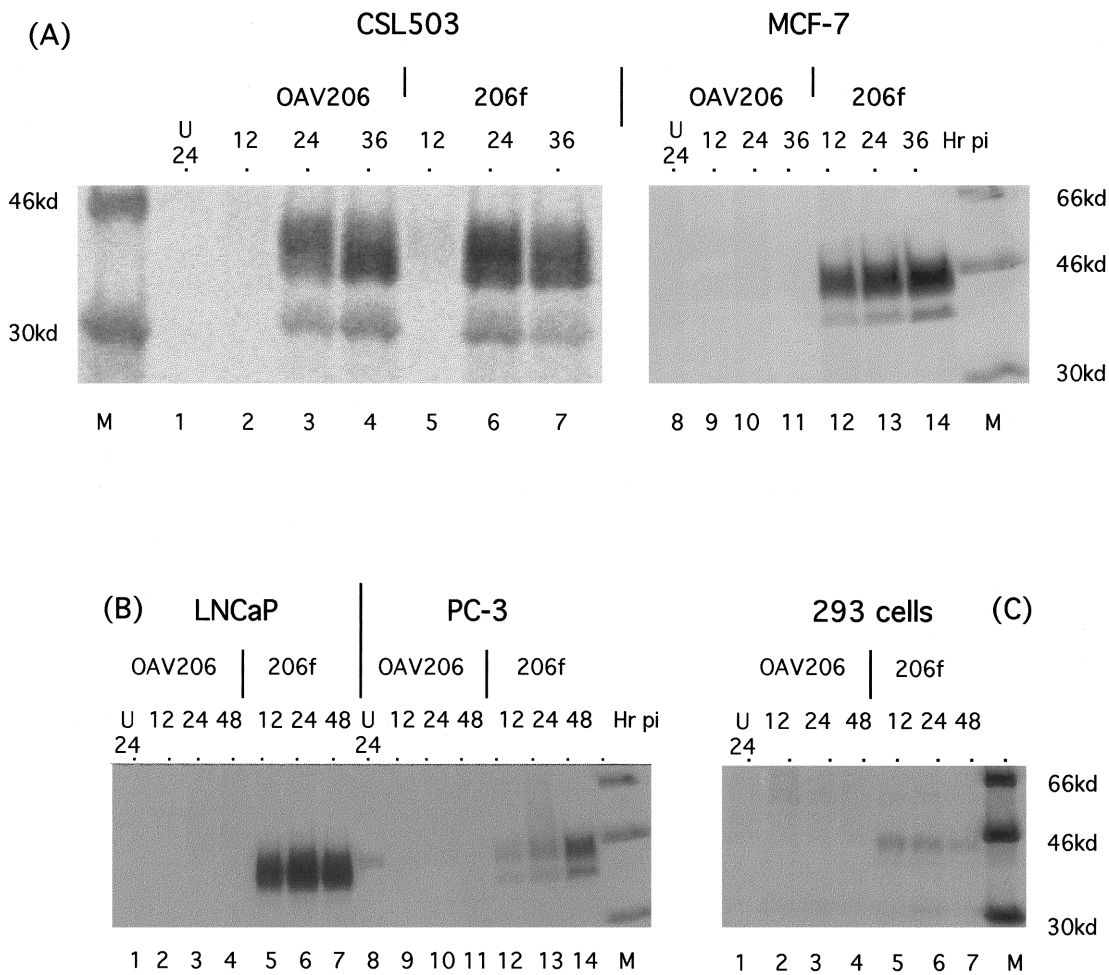

FIG. 5. VP7sc expression in various human cell types is greatly increased by infection with OAV206f. Cells were left uninfected (U) or were infected with OAV206 or OAV206f at an m.o.i. of 10 pfu/cell, radiolabelled and harvested at 12 to 48 h p.i., as indicated. VP7sc was recovered by immunoprecipitation and analysed by SDS-PAGE. (M) Lanes contain marker proteins of the sizes indicated.

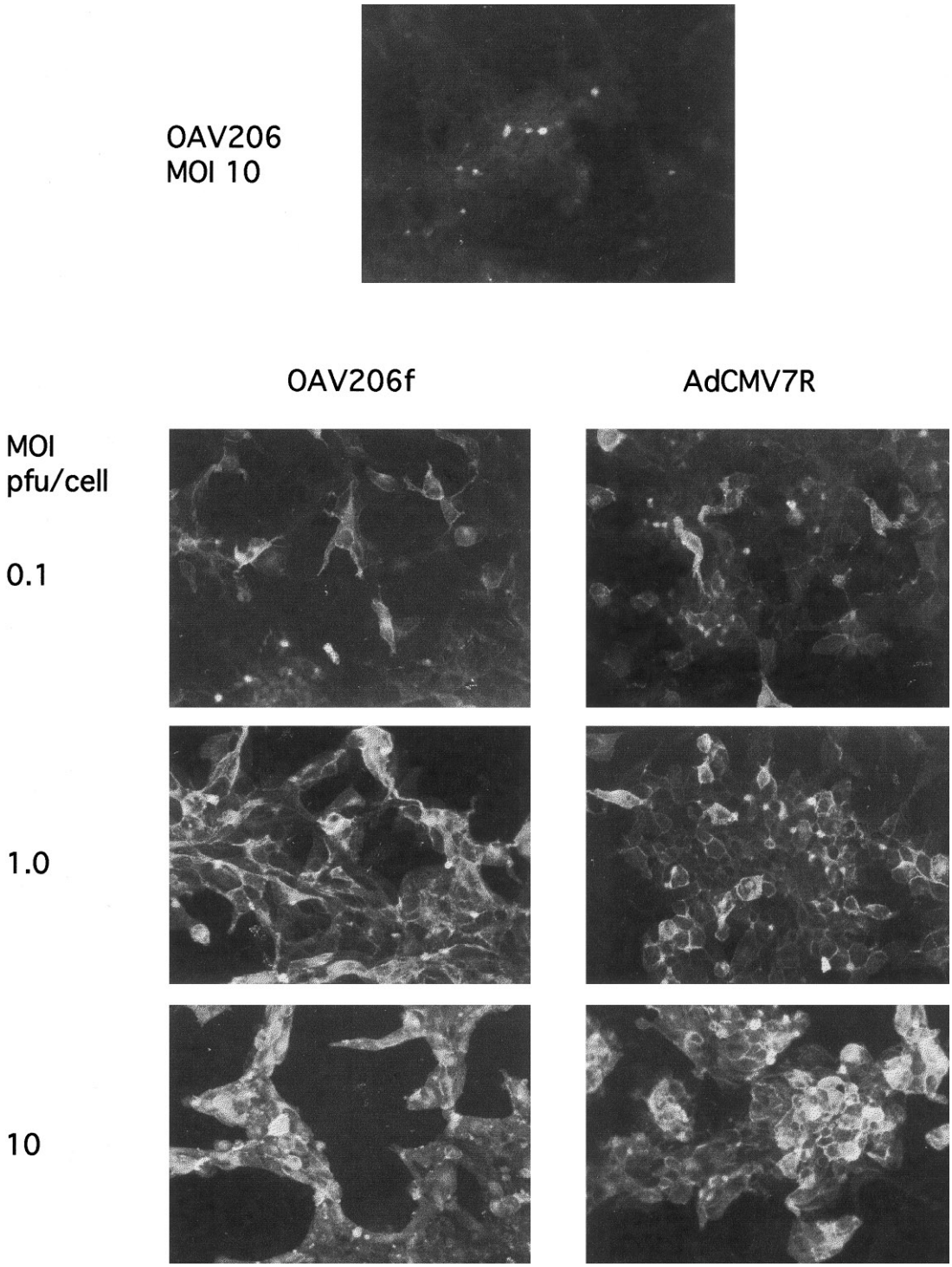

FIG. 6. Expression of VP7sc in OAV206f-infected LNCaP prostate cancer cells. Cells were infected at with OAV206 at an m.o.i. of 10 or with OAV206f or AdCMV7R at m.o.i.s of 0.1, 1, and 10. VP7sc expression was monitored by immunofluorescence at 72 h p.i. The top panel represents a negative control because OAV206 does not infect LNCaP cells.

PC-3 cells (Figs. 5A, lanes 12 to 14, and 5B, lanes 5 to 7 and 12 to 14) and detectably expressed in 293 cells (Fig. 5C, lanes 5 to 7).

The above results for LNCaP cells were confirmed by immuno-fluorescence studies, which also indicated the number of cells that were infected. LNCaP cells infected at an m.o.i. of 10 with the control virus OAV206 showed no significant immunofluorescence, as expected from the above results (Fig. 6, top). However, with OAV206f an increasing number of cells expressed VP7sc after infection at m.o.i.s of 0.1, 1, and 10 (Fig. 6, left). Infectivity by the hybrid virus compared favourably with that of the Ad5

recombinant, AdCMV7R (Xu *et al.,* 1995), which showed similar infectivity in LNCaP cells (Fig. 6, right). Thus, changing the cbd of OAV dramatically improved its ability to infect several nonpermissive cell types and increased its ability to infect a previously recalcitrant cell line to a level comparable with an Ad5 vector.

DISCUSSION

There is considerable interest in modifying Ad cell binding capabilities to develop new vectors that could be targeted to specific cell types. Ads bind to cellular receptors *via* a binding domain formed by C-terminal sequences of the fiber protein (Philipson *et al.,* 1968; Henry *et al.,* 1994; Xia, *et al.,* 1994). Because some receptors are distinct, exchanging appropriate cbds while maintaining the ability of fiber to fold and trimerise should therefore modify viral tropism. Viruses in which the cbd of Ad5 was exchanged for the cbd of Ad7A (Gall *et al.,* 1996) or Ad3 (Stevenson *et al.,* 1995, 1997) have been constructed, and in the latter case, altered cell tropism was demonstrated. In both cases, the size of the substituted cbd was very similar to the cbd that replaced it and little or no difference in the growth properties of the hybrid viruses was noted (Gall *et al.,* 1996; Stevenson *et al.,* 1997).

The present work necessitated a more dramatic modification of the fiber protein because the OAV cbd, which is thought to be ~121 amino acids in size (Vrati *et al.,* 1995), was replaced by the Ad5 cbd comprising ~185 residues. Following transfection of its plasmid (Vrati *et al.,* 1996b), OAV206f was rescued, although cpe appeared more slowly in CSL503 cells than when other viruses were rescued (Khatri *et al.,* 1997; Xu *et al.,* 1997). The growth of OAV206f was also significantly impaired compared with OAV206. The reason(s) for this is not clear but may relate to an impaired rate of virus assembly or exit, rather than entry, because the early kinetics of expression of the transgene in synchronous infections by OAV206f and OAV206 were very similar (Fig. 5A). Several attempts to rescue a second fiber-modified virus carrying an alkaline phosphatase reporter gene cassette have been unsuccessful under conditions where the rescue of other viruses has succeeded (unpublished observations). Perhaps, because of size difference, the exchange of cbds in this work might be close to the tolerable limit. Further work is required to test this.

Competitive binding experiments with OAV206 and OAV206f were carried out to investigate early events in virus binding and uptake by CSL503 cells. A constant amount of radiolabelled OAV206f was competed with increasing amounts of unlabelled OAV206. Preparations of these viruses contained similar numbers of particles as determined by SDS polyacrylamide gel analysis (Fig. 4), but OAV206 competed inefficiently with OAV206f, demonstrating that these two viruses carried distinct cbds. The particle to pfu ratio of the Ad5 virus preparation was not determined, however, Ad5 competed efficiently with OAV206f, consistent with the fact that at the genetic level the viruses were designed with a common cbd.

OAV penton lacks an RGD domain (Vrati *et al.,* 1996a), and it is not known whether a secondary receptor is involved in OAV uptake. Nevertheless, we also attempted to rescue a hybrid OAV in which RGD sequences (102 amino acids) from the Ad5 penton were inserted at residue 247 of OAV penton i.e., LTKGNISPLL-DVDAYQSLK. . .43aas. . . RGD. . .39aas. . . KPQKKPVL-EPVM QDEN GVSYNEKIS (OAV sequences are shown in bold). However, rescue of this virus was also unsuccessful. A smaller insertion, e.g., the equivalent sequences from Ad12, may have been more appropriate but has not been attempted because it seems that the primary receptor is more important for OAV uptake, as discussed below. Other integrin-binding motifs that have been described for cell adhesion proteins (Hynes, 1992) are also absent from OAV structural polypeptides, with the exception of DGEH, a sequence that is closely related to DGE(A), which binds $\alpha 1\beta 1$ integrin (Hynes, 1992). DGEH is present within p28K, an OAV structural protein whose position in the viral capsid is unknown (Vrati *et al.,* 1996a). The significance of this observation being uncertain, it seems likely that OAV may enter cells *via* a single receptor, unless other undefined viral sequences bind integrin or another cellular protein.

Recently two primary receptors for Ad5 were identified on HeLa cells. CAR, a protein that acts as a receptor for coxsackie B viruses and adenovirus types 2 and 5 (Ad2, Ad5) was described (Bergelson *et al.,* 1997; Tomko *et al.,* 1997), and an alternative binding site for Ad5 was also identified on the conserved $\alpha 2$-domain of MHC-I complexes (Hong *et al.,* 1997). Competitive binding experiments carried out in this work confirmed that the OAV and Ad5 receptors on CSL503 cells are distinct. Although it is unknown whether OAV requires a secondary receptor for infection, our data suggest that the interaction between the primary receptor and the virus particle is the major factor controlling virus entry during infection. In the absence of other changes, the inability of OAV to infect 293 and LNCaP cells was overcome simply by substituting the Ad5 cbd, and, in addition, MCF-7 and PC-3 cells were more efficiently infected by the hybrid virus than by OAV206. (Because OAV206 and OAV206f carried the identical promoter/gene cassette, the data for a given cell type cannot be explained by transcriptional or translational differences). The simplest interpretation of these results is that OAV206f infected 293 and LNCaP cells because the Ad5 receptor was present (both lines were infected by Ad5) while the OAV receptor was not, whereas for PC-3 and MCF-7 cells the Ad5 receptor was present in greater numbers. The reduced ability of OAV206f to infect 293 compared with LNCaP cells may also reflect a difference in Ad5 receptor numbers between these cell types. Alternatively, efficient infection of

293, but not LNCaP cells (and other cell types) may require interaction with a secondary receptor such as integrin, as typified by Ad5 (Wickham *et al.,* 1993). OAV lacks such an integrin-binding domain (Vrati *et al.,* 1996a).

Infection of human cells by OAV206 and OAV206f mimics the situation described for infection of A549 cells by Ad2 mutants which lack the penton RGD motif (Freimuth, 1996). In this case, it was demonstrated that virus internalisation was receptor concentration dependent, entry being facilitated by recruitment of multiple receptors. It now seems possible, given the emerging diversity of receptors (Bergelson *et al.,* 1997; Hong *et al.,* 1997; Tomko *et al.,* 1997), that the virus could bind one or more fiber protein receptors on the cell surface. As long as one protein was recycled to the endosomes by endocytosis, the virus would achieve entry. In addition, no particular spatial arrangement between primary and secondary receptors is required. It was recently demonstrated that infection by fiber antibody-adapted Ads could occur via nonadenovirus receptors and that the binding and subsequent entry steps were not linked (Douglas *et al.,* 1996; Wickham *et al.,* 1996). Similar adaptation of OAV should produce a targeted virus that would retain its abortive replication properties in the cell (Khatri *et al.,* 1997). However, the efficiency and kinetics of uptake via a single receptor need to be considered.

MATERIALS AND METHODS

Viruses and antisera

Human adenovirus type 5 (Ad5) was obtained from ATCC. An Ad5/RSV/lacZ recombinant that expressed β-galactosidase from the Rous Sarcoma Virus (RSV) promoter was obtained from Dr. M. Perricaudet (Inst Gustave Roussy, CNRS, France). AdCMV7R, an Ad5 recombinant that carried a reporter gene cassette (HCMV/VP7sc) in which the rotavirus VP7sc gene was expressed from the human cytomegalovirus (HCMV) promoter, was constructed previously (Xu *et al.,* 1995). The origin of OAV287 (OAV) has been described (Boyle *et al.,* 1994). OAV206 and OAV216 are recombinant ovine adenoviruses that express VP7sc or human placental alkaline phosphatase, respectively, from the HCMV promoter (Khatri *et al.,* 1997), but are otherwise identical and have a wild-type fiber protein. Polyclonal antiserum to Ad5 was purchased from ATCC. Polyclonal antiserum against OAV was raised in sheep as previously described (Boyle *et al.,* 1994).

Cell lines

The following cell lines were used in this work: CSL503, embryonic ovine lung fibroblast; and human lines, 293 embryonic kidney; LNCaP, prostate cancer; PC-3, prostate cancer and MCF-7, breast cancer. Their source and culture conditions were described previously (Khatri *et al.,* 1997).

Construction of an OAV/Ad5 hybrid virus

Strategies for the construction of OAV recombinant plasmids and the rescue of infectious viruses have been described previously (Vrati *et al.,* 1996b). Plasmid pOAV206 contains a reporter gene cassette, HCMV/VP7sc, inserted at site I in the intergenic region between the pVIII and fiber genes. This plasmid was further modified to replace the sequences encoding shaft repeat units 24 and 25 (the last two repeats) plus the cbd of the OAV fiber protein with the equivalent sequences from human Ad5 (Henry *et al.,* 1994). An ~7.2-kb *Sph*I/*Sal*I fragment of OAV that contained the fiber gene and adjacent sequences was subcloned into *Sph*I/*Sal*I-cut plasmid pAlter-1 (Promega, Madison, WI). Mutagenic oligonucleotides and the Altered Sites II mutagenesis kit (Promega) were used to introduce unique restriction sites at the appropriate locations in the fiber gene. The introduction of an *Nco*I site in repeat 24 of OAV fiber (Vrati *et al.,* 1995) altered the amino acid sequence from NALTLN to NAMVLN. The C-terminal sequence AHQFRQ was changed to AHQARQ by introducing a *Cel*II site. Oligonucleotide primers incorporating *Nco*I and *Cel*II sites were used to PCR-amplify the equivalent portion of fiber in the Ad5 genome from the MVPKLG sequence in repeat 21 to the C terminus. This PCR fragment was digested with *Nco*I and *Cel*II and cloned into the *Nco*I/*Cel*II-cut recombinant pAlter-1 plasmid to replace the OAV sequences. A ~6.2-kb *Age*I/*Sal*I fragment containing the hybrid fiber gene was then subcloned into *Age*I/*Sal*I-cut plasmid pOAV206 to create pOAV206f. The sequence of the relevant parts of the hybrid fiber protein is shown in Fig. 2.

Virus rescue, preparation, and titration

Plasmid pOAV206f was digested with *Kpn*I to release the linear viral genome and transfected into CSL503 cells using lipofectamine (GibcoBRL) as described previously (Vrati *et al.,* 1996b). A cytopathic effect appeared in one of eight 60-mm dishes of cells after 3 weeks. The virus rescued was grown and characterised by restriction enzyme digestion to confirm that its genome structure was correct. Virus stocks were grown in CSL503 cells.

Ad5 and OAV206f viruses were grown in 293 or CSL503 cells, respectively. To prepare radiolabelled viruses, cells at ~80% confluency in a 100-mm dish were infected at an m.o.i. of 5 pfu/cell. After 16 to 24 h, cells were incubated in 5 ml of serum-free medium containing 0.2 mCi of ^{3}[H]-thymidine (ICN, Irvine, CA). After 8 h, 8 ml of medium was added. Cells were harvested at 48 to 80 h p.i. in 0.1 M Tris.HCl, pH 8.0. Virus was released by three freeze/thaw cycles and purified by CsCl gradient centrifugation as previously described (Boyle *et al.,* 1994).

Virus binding assays

Binding of viruses to CSL503 or 293 cells was carried out as follows. Approximately 5×10^4 cells/well were grown in a 96-well tissue culture plate. Cells were washed once with binding medium (MEM supplemented with 20 mM HEPES and 2 mM $MgCl_2$). Duplicate wells were pre-incubated in binding medium with increasing amounts of unlabelled virus for 2 h at 4°C. A fixed amount of ^{3}[H]-labeled virus was added and the cells were incubated for 1 h at 4°C. The inoculum was then removed and the cells were washed three times with PBS containing 0.2% Triton-X100. Cells were solubilised and radioactivity was determined by liquid scintillation counting.

Infection of cells and expression of antigens

CSL503 and human cell lines were mock infected or infected with viruses at multiplicities of infection of 0.1 to 20 pfu/cell as described previously (Xu *et al.,* 1995). Infection was allowed to proceed for 24 to 72 h. Cells were then incubated in methionine-free medium in the presence of [^{35}S]methionine to label newly synthesized proteins. VP7sc was recovered from cell lysates by immunoprecipitation using a rotavirus antiserum (Xu *et al.,* 1995). Proteins were analysed by polyacrylamide gel electrophoresis and detected by autoradiography or by using a phosphorimager (Molecular Dynamics). Alternatively, VP7sc expression was monitored by immunofluorescence as described previously (Khatri *et al.,* 1997)

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